
BIOREMEDIATION OF TOXIC METALS FROM POLLUTED SOIL USING ARBUSCULAR MYCORRHIZAL FUNGI

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ABSTRACT

This study investigated the potential of *Amaranthus spinosus* and Arbuscular Mycorrhizal (AM) fungi in bioremediating toxic metals. Pot experiment was conducted. *Amaranthus spinosus* was grown in soil contaminated with toxic metals, with and without AM fungal inoculation and grown for eight weeks. Atomic Absorption Spectrophotometer (ASS) was used to determine the levels of Fe, Cd, Cr and Pb in the soil samples before planting and after harvest and in the harvested *Amaranthus spinosus* test plant. Data obtained were analyzed using analysis of variance (ANOVA) and means were separated using the least significant difference (LSD) test at $p < 0.05$. The results revealed that AM FUNGI significantly ($p < 0.05$) reduced the uptake of Fe, Cd and Pb by *Amaranthus spinosus* and immobilized Fe by 52.23% , Pb by 71.94% and Cd by 43.33% thereby reducing their availability for uptake by *Amaranthus spinosus*. The combination of *A. spinosus* and AM fungi resulted in a significant decrease in soil metal concentrations. This study highlights the potential of *A. spinosus* and AM fungi as a sustainable and eco-friendly tool for enhancing soil resilience and reducing the uptake of heavy metal by plants grown on toxic metal polluted soil.

Keywords: Bioremediation, *Amaranthus spinosus*, Arbuscular Mycorrhizal fungi, toxic metals, *Polluted soil*

INTRODUCTION

Deposition of Heavy metal in the environment poses a serious threat to biodiversity and human health due to their toxicity and non-biodegradability, a wide range of pollutants, such as heavy metals, polychlorinated biphenyls, plastics, and various agrochemicals are present and persistent in the environment. Soil contamination with heavy metals is detrimental to the biological process in the environment (Enerijiofi, 2021). Plants grown on heavy metal contaminated soil could act as an intermediate reservoir for transferring metals from soil to human being and other animals (Zhou *et al.*, 2022; Silva *et al.*, 2019). Accumulation of some of these metals in plants, could impact negatively some physiological activities like photosynthesis, mineral and water transport.

Several chemical and physical methods have been employed for the clean-up of heavy metals from the environment. These methods have major limitations such as high cost, high energy consumption, and negative impact on the chemical properties of the soil and inefficiency in removal of metals at low concentration (Tarekegn *et al.*, 2020). Bioremediation offers a more promising alternative to the conventional methods for metal decontamination (Tufail *et al.*, 2022). Microorganisms play a major role in bioremediation by degrading and converting pollutants to less toxic forms, which is a primary goal of bioremediation. However, when plants are used for bioremediation, heavy metals sometimes have toxic effects on the plants. Thus, microorganism mediated bioremediation of heavy metals can be the answer to all the limitations posed by phytoremediation alone. It has been reported that metal-tolerant microbes can excrete various metabolites, which play a significant role in the mobilization of heavy metals in plants (Kebede *et al.*, 2021). Since bioremediation seems to be a good alternative to conventional clean-up technologies; substantial research in this field is rapidly increasing with the purpose of identifying specific organisms with great potentials for clean-up of heavy metal polluted soil (Thakare *et al.*, 2021).

There have been several reports on the bioremediative potential of different bacteria strains (Nguyen *et al.*, 2019; Zhou *et al.*, 2022; Thakare *et al.*, 2021). Several bacterial species such as *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Gluconacetobacter*, and *Serratia* have been reported to solubilize and/or tolerate heavy metals (Del Álamo *et al.*, 2022). Many fungi and bacteria produce secretions that attract metals that are toxic in high levels. Some fungi species have also been proven to have potentials of enhancing bioremediation of heavy metal polluted soil (Akhtar and Amin-ul, 2020). Fungi have been proven to be the most efficient removers of heavy metals because of its relatively higher tolerance to heavy metals, robust nature in comparison to bacteria and larger surface to volume ratio (Bala *et al.*, 2022). Different fungal groups have been used to clean up polluted soil (Akpasi *et al.*, 2023). However, reports on the synergistic potential of *Amaranthus spinosus* and Arbuscular Mycorrhizal Fungi are scarce. This study investigated the capacity of Arbuscular Mycorrhizal fungi in enhancing the phytoremediative ability *Amaranthus spinosus* plant in the clean-up of soil that is metal polluted soil.

MATERIALS AND METHODS

The Study area

The study was carried out in the Botanical garden of Confluence University of Science and Technology, Osara, Kogi State, Nigeria. Geographically, the study area is bounded by latitudes 7° 30' N and 7° 45' N, and longitudes 6° 30' E and 6° 45' E. The site is characterized by a tropical savanna climate, with two distinct seasons: a wet season from April to October, and a dry season from November to March. The mean annual precipitation at Osara is 1,200

mm. The peak period of rain is between July and September with a short break experienced in August. Relative humidity varies from 80% between July and August to 60% between January and February. The monthly average temperature ranges from 28⁰C - 29⁰C in July and August to 34⁰C -36⁰C in February and March. The vegetation of the area is characteristic of the forest Savanna with bush scattered low trees, grasses and shrubs. The soil of the study area is predominantly sandy loam and supports luxuriant growth of tuber and cereal crops.



Fig. 1: Map of Study area showing sampling sites

Source; Google earth

Samples collection

A total of 18 soil samples were randomly collected from sites where heavy metal pollution has been previously reported. Control samples were collected from the Botanical garden of confluence university of Science and technology, Osara, Kogi State. Soil samples were collected in the month of February. Three sub subsamples were collected within an area of 10 m by 10 m with a stainless-steel hand auger sampler and homogenized to make a composite sample. Samples were obtained to a diameter of 3cm and a vertical profile of 0–15 and 15–30 cm in each of the sampling locations and control sites (Ajibade *et al.* 2023).

Certified *Amaranthus spinosus* seeds to be sown on the polluted soil were obtained from Ministry of Agriculture office at Lokoja Kogi state while Arbuscular Mycorrhizal fungi strains were obtained from the microbiology laboratory of University of Ibadan. The experimental plot was in the screen house of the Department of Biology, Confluence University of Science and Technology, Osara, Kogi State.

Determination of soil physicochemical parameters

Collected soil samples were divided into two parts for experimentation. One part (200g) was subjected to physico-chemical analysis. While planting of the test plant and mycoremediation test was carried out on 2kg of each sample collected. The pH of the sampled soil was determined using an ion-selective electrode (ISE) pH meter (Hanna instrument HI5222). The pH meter was calibrated using three standard buffer solutions of pH 4.0, 7.0 and 9.0. Solution of the soil sample was made using distilled water (1:2.5). A 10 ml aliquot of the solution was transferred to a clean dry beaker. The pH electrode was immersed in the sample and the pH reading was allowed to stabilize for 1-2 minutes. The pH value was then recorded to the nearest 0.01 pH unit.

Determination of soil organic matter content

Organic matter content of the soil samples was determined using the loss-on-ignition (LOI) method. Two (2g) of the air-dried soil sample was placed in a pre-weighed ceramic crucible, the sample was then ashed in a furnace at 650⁰ C for 24 hours, after which the sample was re-weighed when it was cool. The soil organic matter content was calculated using the formular:

$$\text{Organic matter (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

-----Reddy *et al.* (2009)

Determination of Electrical Conductivity (EC)

The electrical conductivity of soil samples was measured with a soil:water ratio 1:2 (w/v) using Digital Conductivity meter (Delta-T Device EC-20) as follows: 5g of 2 mm sieved air-dried soil samples was weighed into a beaker and 10cm³ of distilled water added. The mixture was stirred several times. The soil suspension was allowed to stand for 30 minutes. The electrodes of the conductivity meter were then inserted into the settled suspension and the electrical conductivity of the soil measured. Before use, the electrical conductivity meter was calibrated using 0.1M KCl.

Determination of Total heavy metal content and bioavailable heavy metals

i. Digestion of soil and plant Samples

Wet digestion method was used for digestion of the soil samples. Soil samples for determination of Ni, Pb, Cr, Fe, Cd and Co was digested using; Aqua-regia (prepared from 3:1 ratio of analytical grade HCl and HNO₃) (Jaing *et al.*, 2011). One (1) gram of each soil sample was weighed using electric weighing balance into a 250 ml glass conical flask and 10ml of concentrated HNO₃ was added. The mixture was then boiled gently in a fume chamber for 45 minutes. After cooling, 5ml of 70% HCl was added and the mixture boiled again gently until dense white fumes appeared. All the digests were cooled and filtered using a whattman filter paper No. 42 and diluted to 50ml by adding double distilled water. Each digest was then transferred into acid-washed stoppered glass bottle, appropriately labelled and kept for metal analysis. *Amaranthus spinosus* grown on the AM fungi amended soil and those grown on soil which were not amended were all harvested and subjected to similar digestion procedure as enumerated above for soil samples. Quantification of total Ni, Pb, Cr, Fe, Cd and Co in the soil and plant samples was carried out by analyzing the extract with Atomic

Absorption Spectrophotometer (Thermo Scientific Ice 3500). Certified reference material IAEA-SL-1 (lake sediment) and certified reference material IAEA-Cabbage-359 were subjected to same digestion procedure as above and analysed accordingly using same Atomic absorption Spectrophotometer. The percentage recoveries of the standard reference materials were calculated to evaluate the accuracy of the analytical procedure

Inoculation of AM Fungi

Isolates of Mycorrhizal fungi spores was obtained from microbiology laboratory of University of Ibadan. Identification and purity of the fungal isolates was determined using morphological techniques. The inoculum was prepared by mixing the AM fungal spores with sterile carrier material (sand). Two kilograms of each soil sample collected from the study area was weighed into a sterile container. AM fungal spores was added 1-5% (v/v), 1-10% (v/v), 1-15% (v/v) and 1-20 (v/v), The soil and spores was mixed thoroughly to ensure uniform distribution. The inoculated soil was transferred into polypropylene pots. Watering was done to field capacity once every other day. Each inoculation concentration had two replicates. The control pots had no inoculated spores of AM fungi. The seeds of *Amaranthus spinosus* were then sown into each pot after three days of inoculation. The potted plants were arranged in a randomized block design and allowed to grow for three months in a screen house before harvest.

Heavy metal analysis of soil and plant parts

At the time of harvest of the test plant about 5 grams of soil was collected from the rhizosphere region of the plant and processed following the step outlined above for heavy metal analysis. Similarly, one plant from each pot was uprooted and processed for analysis of it heavy metal content of the leaves, roots and stem. All sampling and analysis was carried out in duplicate.

Statistical analysis

Data obtained were subjected to basic statistics and one-way analysis of variance (ANOVA) while means separated using DUNCAN multiple range test to determine the differences among the treatments within respective inoculation concentrations at $p \text{ value} \leq 0.05$. All the statistical analysis was carried out using SPSS software (version 22).

RESULTS AND DISCUSSION

Physicochemical Characteristics

The result of average pH, electrical conductivity and organic matter of the contaminated soil as determined following the procedures mentioned above is presented on (Table 1). The result showed that bioremediation by *Amaranthus spinosus* enhanced by AM fungi had a significant effect on the pH, electrical conductivity and organic matter of the soil ($p < 0.05$). There was a significant difference between the physicochemical characteristics of the soil before bioremediation in comparison to the after-bioremediation values (Table 1). The result showed an increase (before/after bioremediation) in pH (5.92 ± 0.06 to 6.07 ± 0.03) electrical conductivity (486 ± 16.3 to $747 \pm 22.8 \mu\text{S/cm}$) and organic matter content (4.13 ± 1.1 to $4.15 \pm 1.3\%$) of soil sampled from the mountain top which is the very mining site. Soil samples obtained from farmlands that were one kilometer away from the mining site also recorded increased pH (7.04 ± 0.03 to 7.67 ± 0.04) electrical conductivity (406 ± 23.1 to $389 \pm 32.3 \mu\text{S/cm}$) and organic matter content (3.67 ± 0.5 to $4.03 \pm 0.7\%$). The results of heavy metals (Ni, Pb, Cr, Fe, Cd, Co) values of soil samples before and after bioremediation are presented in table 2. It shows that there was a significant ($p < 0.05$) difference in the heavy metal level after the bioremediation experiment.

Table 1: Mean values of pH, EC and OM of soil samples before and after bioremediation

Soil Samples	GPS coordinates	Before/after remediation	pH	EC(μS/cm)	OM (%)
S1	N 07° 36'57.0" E06° 19' 09.4"	Before	5.92±0.06	486±16.3	4.13±1.1
		After	6.07±0.03	747±22.8	4.15±1.3
S2	N 07°36' 27.2" E 6° 19' 06.8"	Before	5.72±0.04	635±31.5	4.71±0.8
		After	6.02±0.07	612±26.4	4.95±1.2
S3	N 07° 37' 16.4" E 6° 19' 10.5"	Before	7.04±0.03	406±23.1	3.67±0.5
		After	7.67±0.04	389±32.3	4.03±0.7
CNTRL	N 07° 37' 16.4" E 6° 19' 10.5"	Before	6.88±0.05	437±40.2	4.11±1.0
		After	6.94±0.02	449±34.5	4.82±1.1

Key: S1-S3; Composite Samples 1-3.

Table 2: Heavy metal levels (mg/kg) of soil before and after Bioremediation

Sample Codes	GPS coordinates	B/A	Ni	Pb	Cr	Fe	Cd	Co
S1	N 07° 36' 34.5" E 006 17' 51.8"	B	36± 2.8 ^b	213± 12.8 ^b	57±4.8 ^a	1585±34.6 ^c	5.76±1.8 ^a	32.34±4.5 ^b
		A	12± 2.8 ^d	104±23.5 ^{bc}	54±5.2 ^a	1397±52.4 ^c	2.34±0.4 ^c	28.94±4.2 ^b
S2	N 07° 36' 51.3" E 06 19' 55.3"	B	53 ± 2.8 ^a	188±20.3 ^b	55±3.9 ^a	1251±41.0 ^d	4.04±1.6 ^b	32.58 ±3.8 ^b
		A	22± 2.8 ^c	103±18.6 ^c	51±7.3 ^a	1200±22.8 ^d	1.34±0.2 ^c	31.46±5.3 ^b
S3	N 07° 36'57.0" E06° 19' 09.4"	B	43± 3.2 ^{ab}	256±26.8 ^a	50±4.9 ^a	1576±26.9 ^c	5.04±1.7 ^a	40.27±5.8 ^a
		A	20± 2.8 ^c	111±10.7 ^{bc}	50±2.7 ^a	1396±39.4 ^c	1.45±0.5 ^c	36.38±2.8 ^a
S4	N 07°36' 27.2" E 6° 19' 06.8"	B	50 ± 2.0 ^a	255±29.5 ^a	47±5.8 ^a	1441±38.0 ^c	3.03±0.7 ^c	43.08 ±6.3 ^a
		A	21± 4.8 ^b	173±12.7 ^b	43±4.6 ^b	1429±27.6 ^c	2.19±0.5 ^c	40.67±4.9 ^a
S5	N 07° 37' 34.9" E 6° 19' 08.6"	B	57± 4.1 ^a	267±24.6 ^a	27±2.7 ^c	2545±37.6 ^a	4.11±1.8 ^b	20.18 ±6.1 ^c
		A	20± 2.2 ^c	136±16.4 ^{bc}	25±3.2 ^c	2458±49.3 ^a	2.03±0.4 ^c	20.16±3.7 ^c
S6	N 07° 38' 06.4" E 006 20' 12.5"	B	53± 3.8 ^a	294±12.4 ^a	40±3.8 ^b	2711±38.5 ^a	3.65±0.7 ^b	25.44±4.1 ^b
		A	25± 2.4 ^c	128±16.4 ^c	37±6.3 ^b	2643±31.0 ^a	1.56±0.5 ^c	21.56±3.8 ^c
S7	N 07°36' 35.4" E 6° 19' 12.8"	B	54± 2.9 ^a	276±22.6 ^a	39±3.1 ^b	2611 ±35.1 ^a	4.22±1.2 ^a	34.55±5.1 ^b
		A	26± 1.8 ^b	104±17.4 ^c	37±4.0 ^b	2532±32.7 ^a	1.11±0.5 ^d	28.49±3.0 ^b
S8	N 07° 37' 16.4" E 6° 19' 10.5"	B	46± 3.1 ^a	202±22.8 ^b	42±3.2 ^b	1842±22.8 ^b	3.67±1.3 ^b	42.81±3.7 ^a
		A	21± 2.4 ^b	101±9.5 ^c	40±4.1 ^b	1800±31.7 ^b	1.54±0.7 ^c	37.49±2.9 ^a
S9	N 07° 38' 06.4" E 006 20' 12.5"	B	41± 2.5 ^{ab}	249±20.5 ^{ab}	49±4.8 ^a	2056±21.6 ^b	4.86±1.3 ^a	37.92±3.8 ^a
		A	18± 3.8 ^c	105±11.7 ^c	44±6.3 ^b	2005±27.9 ^b	2.02±0.7 ^c	34.90±4.0 ^b
CNTRL	N 07° 38' 06.4" E 006 20' 12.5"	B	38± 3.5 ^b	152±12.4 ^b	37±3.3 ^c	953±27.3 ^d	3.78±0.2 ^b	20.03±4.2 ^c
		A	22± 2.6 ^b	98±8.3 ^c	34±4.7 ^c	942±22.6 ^d	1.67±0.8 ^c	18.99±2.7 ^c

Means with different subscripts are significantly different (p < 0.05) and separated using DMRT

Key: S1-S9; Samples 1-9. B-Before bioremediation, A- After bioremediation

The results of this study demonstrate the potential of using *Amaranthus spinosus* and Arbuscular Mycorrhizal (AM) fungi for enhanced phytoremediation of toxic metals from contaminated soils. Bioremediation of the sampled soil using *Amaranthus spinosus* and Arbuscular Mycorrhizal (AM) fungi increased the pH of the soil. This recorded increase in pH is attributable to the ability of AM fungi to stabilize soil pH by increasing the cation exchange capacity (CEC) and buffering capacity of soil. This can lead to a slight increase in soil pH. Increased soil pH owing to bioremediation agrees with findings of Dou et al., 2024 who reported a marginal increase in soil pH from acidic range to alkaline range through AM fungi soil amendment. One of the most concerned factors that command the conversion of metals from immobile-solid phase to more easily bioavailable forms is pH of the soil (Kebede *et al.*, 2021). It is presumed that at higher pH values, less toxic metal availability is detected. Apart from metal bioavailability, soil pH also affects the process of metal uptake into roots though this is metal specific (Bilal *et al.*, 1995).

Implications for Environmental Remediation

The results of this study have significant implications for environmental remediation. The use of *Amaranthus spinosus* and AM fungi for phytoremediation provides a sustainable and eco-friendly solution for the cleanup of contaminated soils. This approach is particularly useful for remediation of sites contaminated with multiple toxic metals, as *Amaranthus spinosus* and AM fungi can simultaneously remove multiple metals from soil.

Limitations and Future Directions

While the results of this study are promising, there are several limitations that need to be addressed in future studies. For example, the study was conducted under controlled laboratory conditions, and further research is needed to evaluate the effectiveness of *Amaranthus spinosus* and AM fungi under field conditions. Additionally, the long-term sustainability of this approach needs to be evaluated, as well as the potential risks associated with the use of AM fungi.

Conclusion

In conclusion, the results of this study demonstrate the potential of using *Amaranthus spinosus* and Arbuscular Mycorrhizal fungi for enhanced phytoremediation of toxic metals from contaminated soils. The synergistic effect of *Amaranthus spinosus* and AM fungi on phytoremediation provides a sustainable and eco-friendly solution for environmental remediation. Further research is needed to evaluate the effectiveness and sustainability of this approach under field conditions.

Author contributions

Study conceptualization and methodology design: OVA, investigation: OVA and OTT, formal analysis: OVA, manuscript original drafting: OVA, manuscript review and editing: OVA and OTT.

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Declarations Conflict of interest

No potential conflict of interest was reported by the author(s).

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